

## BACKGROUND

Myosins are a large family of motor proteins that share the common features of ATP hydrolysis, actin binding and potential for kinetic energy transduction. One myosin molecule consists of two heavy chains and two pairs of light chains. The light chains stabilize the  $\alpha$ -helical neck region of myosin and are located in close proximity to the myosin ATP binding and actin binding domains. Apart from the structural role, myosin light chains are assumed to modulate and regulate actin-myosin interaction in striated muscle. There are 17 myosin families and the best characterized is myosin II. Myosin II is found predominantly in myocytes and mediates plus-ended movement along microfilaments. It is involved in muscle contraction through cyclic interactions with actin-rich thin filaments, creating a contractile force. Myosin light chains (Myls) are the principal components in myofibrils and associated with myosin heavy chain heads. According to the conditions dissociated from the myosin heavy chains, Myls are divided into two classes. One is called the regulatory (or phosphorylatable) light chain (i.e. Myl2 or RLC) and the other is the alkali light chain (i.e. Myl1, Myl3 and Myl4) or essential light chain (ELC). Each class has several isoforms associated with different muscle types.<sup>1</sup>

Myosin movement can be regulated by phosphorylation of the regulatory light chain of myosin (RLC). This RLC is phosphorylated by  $Ca^{2+}$ /calmodulin-dependent myosin light chain kinase (MLCK) or PKC at Ser19 of RLC, which resulted in increased actin-stimulated myosin MgATPase activity. The phosphorylation also increased  $Ca^{2+}$ -stimulated myofibrillar MgATPase activity upon substitution of the phosphorylated myosin into myofibrils. This will enable the myosin crossbridge to bind to the actin filament and allow contraction to begin (through the crossbridge cycle). Thus, phosphorylation of RLC by  $Ca^{2+}$ /calmodulin-dependent MLCK is a critical step in the initiation of smooth muscle and non-muscle cell contraction.<sup>2</sup> In addition, Myosin regulatory light chain (RLC) phosphorylation has been implicated in Rho-mediated stress fibre formation. It was reported that gamma-PAK, which is activated by the GTP-binding proteins Cdc42 and Rac, catalyses phosphorylation of intact non-muscle myosin II and isolated recombinant RLC. Phosphorylation is  $Ca^{2+}$ /calmodulin-independent and Ser-19 is the only phosphorylation site modified by gamma-PAK. Similar to MLCK, Arg-16 is required for interaction of gamma-PAK with the substrate. It was suggested that myosin II activation by the p21-activated family of kinases may be physiologically important in regulating cytoskeletal organization.<sup>3</sup>

In addition, MLC2 participates in various cell signaling regulations in non-muscle cells. It is known that cells exert force propelling the cell forward by contraction of the actin cytoskeleton

through activation of myosin II. The actin-myosin II interaction in nonmuscle cells is regulated by the phosphorylation of MLC2 at serine-19 too. MLC2 dephosphorylation can induce apoptosis and inhibitor of MLCK can abrogate MLC2 phosphorylation, cell polarization and migration. MLC2 is also involved in the activation of mid-G1 phase cyclin D1 expression. It has been reported that hyperphosphorylated MLC2 induces stress fiber formation and integrin clustering that link cell surface cytoskeletal proteins such as FAK to actin and activates FAK downstream signaling.<sup>4</sup>

## References:

1. Warrick, H.M. & Spudich, J.A. et al: Ann. Rev. Cell Biol. 3:379-421, 1987
2. Noland, T. A. & Kuo, J.F.: Biochem. Biophys. Res. Commun. 193:254-60, 1993
3. Yoneda, A. et al: J. Cell Biol. 170:443-53, 2005
4. Reik, K et al: J. Biol. Chem. 283:35598-605, 2008

## TECHNICAL INFORMATION

### Source:

MLC2 antibody is a rabbit antibody raised against a short peptide from human MLC2 sequence.

### Specificity and Sensitivity:

This antibody detects endogenous MLC2 proteins without cross-reactivity with other family members.

**Storage Buffer:** Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

### Storage:

Store at  $-20^{\circ}C$  for at least one year. Store at  $4^{\circ}C$  for frequent use. Avoid repeated freeze-thaw cycles.

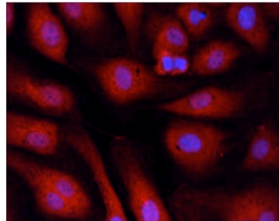
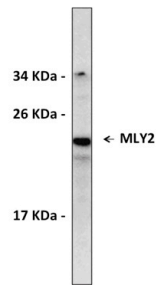
## APPLICATIONS

Application:	*Dilution:
WB	1:500-1:1,000
IP	n/d
IHC	n/d
ICC	n/d
FACS	n/d

*\*Optimal dilutions must be determined by end user.*



## QUALITY CONTROL DATA



**Top:** Lysate of HEK-293T cells overexpressing human MYL2 was separated on SDS-PAGE, blotted with Anti-MYL2 (61-75) and developed using Goat Anti-Rabbit IgG-Peroxidase and a chemiluminescent substrate.

**Bottom:** Immunofluorescence of HUVEC cells using Anti-MYL2 (61-75), SAB1100278 (red), taken at 40× magnification and nuclear staining with Hoescht 33342 (blue).

